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USE OF PHYTOCHROME-DEPENDENT REACTION IN EVALUATING THE EFFECT OF SPACE FLIGHT FACTORS ON THE PLANT ORGANISM

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16. Abstract

This article provides the results of a study on the effects of space flight factors on lettuce seeds aboard the "Kosmos-936" and "Kosmos-1129" satellites for 20 days. The phytochrome--dependent (PD) reaction of light-sensitive seeds was found to be a sensitive criterion for evaluating the biological effects of space flight factors. The PD reaction of air-dry lettuce seeds was suppressed after space flight, and most markedly so if the seeds were exposed to open space during the flight. Space flight affects the physiological activity of both phytochrome forms, as both the Φ_{660}- and Φ_{730}-dependent reactions of lettuce seeds were suppressed.

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USE OF PHYTOCHROME-DEPENDENT REACTION IN EVALUATING THE EFFECT OF SPACE FLIGHT FACTORS ON THE PLANT ORGANISM

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Over the relatively brief period that biological research has been /94* done in space, a great deal of data has been accumulated on the effects of space flight factors (SFF) on various organisms. Most of this material deals with warm-blooded organisms. Relatively little data is available in the literature on the effects of SFF on air-dry seeds of higher plants. Plant seeds are convenient for use in space research to reveal the biological effects of SFF on plants. Data on the effects of SFF on plants would be useful in long duration satellite flights.

This article deals with a study of SFF effects on plants. The phytochrome-dependent (PD) reaction of lettuce seeds was used as a criterion for this purpose. This reaction is based on activation of diverse physiological processes following irradiation of light-sensitive seeds with red light. These processes are activated by a special sensitizer -- phytochrome. As an example emphasizing the diversity of plant reactions to exposure to red light, we can enumerate the following physiological processes controlled by the phytochrome system: seed germination [1], plant growth [2], enzyme activity [3, 4], change in /95 membrane properties [5], gene DNA derepression [6, 7], etc. The PD reaction is characteristic of several types of plant seeds (lettuce, tomatoes, sorrel, pine, etc.).

The PD reaction depends on the conditions surrounding the seeds, and thus can be used to reveal the biological effects of various environmental factors. We have found that the PD reaction is more pronounced after lettuce seeds are irradiated with sublethal doses of gamma-rays [8]. Virtually the same effect has been found by other investigators as well [9].

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*Numbers in the margin indicate pagination in the foreign text.

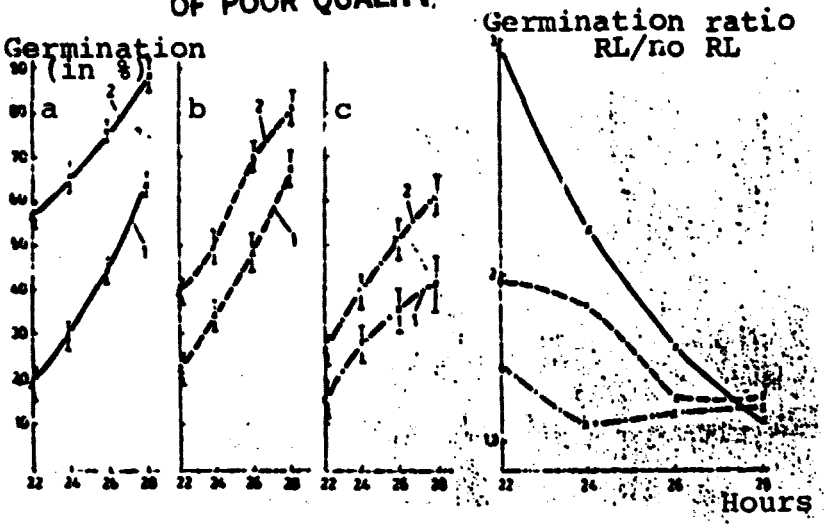
The PD reaction was used in this work as a criterion for evaluating the effects of SFF on plants, i.e., to find out the extent that space flight factors alter the activating effects of red light.

Air-dry seeds of "large-head" lettuce (Lactuca sativa L.) from the 1978 harvest served as the subject of our study. The seeds were exposed to space flight on two satellites, "Kosmos-936" and "Kosmos--1129," for 20 days. In order to make a more detailed study of the biological effects of cosmic rays, during space flight the seeds under study were subjected to two different doses of cosmic rays, this being achieved by using screens of two different thicknesses. An external container with half of the seeds automatically opened after orbital insertion, and in both flights the seeds were for all practical purposes exposed to open space (protective foil 0.02 g/cm^2). During this time, the other half of the seeds was kept in a container within the satellites (screen mass 0.2 g/cm^2). According to thermoluminescent dosimeters, the associated integral dosage of cosmic rays amounted to 0.6 and 0.3 rads in the external and internal containers, respectively.

Biological analysis of the data was carried out at the Latvian SSR Academy of Sciences, Institute of Biology.

A methodology we had previously developed for determining the phytochrome-dependent reaction [8], the basis of which is given in Ikuma and Thimann's article [10], was utilized in our research. The seeds under study were scattered on moist filter paper in flat trays, 20 seeds each in 5 repetitions, and placed in a constant-temperature cabinet at +26°. 2 hours after soaking, the seeds were for 3 minutes illuminated with 660-nm or 730-nm light at 6×10^{11} $erg\text{-}cm^{-2}$ sec^{-1}. All operations were carried out in the soft green light emitted by an incandescent bulb with 2 filters: dark green and yellow.

To define primary root growth, the sprouts were placed between two layers of rolled-up filter paper, which, in turn, were placed in a container with tap water. Primary root length was determined on the 5th, 7th, and 10th days of plant development. On the 10th day the



a -- control
b -- internal container
c -- external container
1 -- without red light
2 -- with red light

Fig. 1. PD reaction change during lettuce seed germination relative to different spacecraft screen thickness.

sprouts were planted in containers with quartz sand, using Knop's nutrient solution. The plants were grown in a greenhouse at a temperature of 25° under fluorescent lamps. On the 30th day of plant growth the accumulated biomass was analyzed for the following: number of leaves, leaf and root raw and dry mass, leaf dry matter content, total photosynthetic productivity (dry leaf mass/leaf area, mg/100 cm^2).

Data on the growth dynamics of lettuce seeds exposed on the /96
"Kosmos-1129" satellite showed that the seeds from the internal container differed little from the laboratory control (fig.1, a, b, curve 1). The growth of the seeds kept in the external container, however, was about 25% below that of the control (fig. 1, a, c, curve 1).

After treating the experimental seeds with red light (RL) it was found that SFF suppress the PD reaction. This was especially true after the seeds were exposed to open space. These discrepancies gradually diminished in later growth processes. On the 7th day, primary root growth continued to show a significant difference between PD reactions in the control and flight versions, which disappeared in later growth. None of the criteria under study demonstrated significant PD reaction discrepancies on the 30th day (table 1).

TABLE 1. LETTUCE PLANT BIOMASS AS AN INDEX OF PD REACTION CHANGE EFFECTED BY SPACE FLIGHT FACTORS

Experimental version (on 30th day cf growth)		Dry leaf mass, mg	Dry root mass, mg	Root dry matter content	Total photosyn. prod., mg/100 cm^2
Control	w/o RL	130,0±10,0	23,0±2,1	5,0	7,2
	with RL	130,0±9,0	26,0±2,5	5,0	8,8
Internal container	w/o RL	120,0±13,0	28,0±3,0	1,8	2,3
	with RL	110,0±8,0	26,0±2,8	5,0	2,1
External container	w/o RL	34,9±1,7	5,7±0,7	9,2	2,7
	with RL	27,9±8,5	5,7±1,1	8,2	3,1

Note: Indices averaged for one plant.

It has recently been suggested that air-dry seeds are of little use in space research [11]. The data we obtained show that lettuce seeds are extremely sensitive. For example, after the seeds were exposed to open space, the dry leaf and root mass of the plants comprised only 26% of the control version mass (table 1). Open space also significantly suppressed seed germination (fig. 1) and primary root growth. Significant biological process suppression thus was noted at an integral dosage of only 0.6 rads.

To clarify the unique effects of the PD reaction in seeds, after space flight we tested the effect of substituting Φ_{660} for Φ_{730} and vice versa in the experimental seeds. The PD reaction occurs as follows under normal conditions: absorbing red light rays, the "red" phytochrome is transformed into "far-red" phytochrome, with a maximum absorption in the region of 730 nm. This form is active, and its presence determines the physiological effect found after exposure to red light. If we designate the two pigment forms and their maximum absorption using the letter Φ (Φ_{660} and Φ_{730}) we can write the relationship between the processes:

$$\Phi_{660} \leftarrow \frac{h\nu 660}{h\nu 730} \rightarrow \Phi_{730}.$$

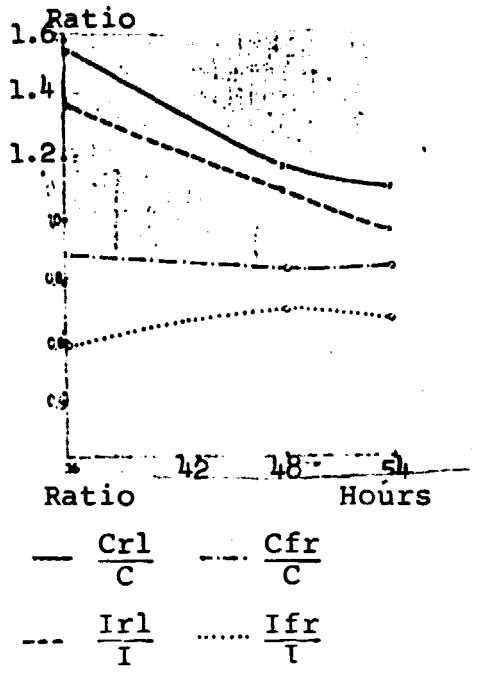


Fig. 2. Effect of red (rl) and far-red (fr) light on the PD reaction of lettuce seeds in internal container (I) on "Kosmos-936."

Absorbing a 660-nm light quantum, a molecule of pigment Φ_{660} is transformed into the physiologically active form, Φ_{730}. When it absorbs a 730-nm light quantum, the latter is transformed into the inactive form, Φ_{660}. /97

We have shown (fig. 2) that the PD reaction of lettuce seeds exposed to space is diminished relative to the control, both with the use of red light and far-red light. Experiments were done in which after space flight the seeds were sequentially irradiated with red and far-red light (table 2). It turned out that the last type of radiation given to the seeds fell behind in strength. Data from these experiments and fig. 2 indicate that the phytochrome transformations are retained in the lettuce seeds exposed to space.

This experiment, like our previous ones [12], shows that the effectiveness of red light on plants diminishes after the seeds are exposed to space. This is evidence that in the flight version seeds some kind of change occurred in the red-light acceptor -- plant response chain. We might assume that SFF somehow affect the transfer of information from phytochrome to cell nucleic acids. This is corroborated by the fact that transcription-blocking inhibitors also suppress the PD reaction [13, 14].

We succeeded in establishing that the PD-reaction changes following space flight are associated with certain changes in the cell genome. Seeds exposed on the "Salyut-6" orbital station (flight duration -- 185 days) manifested not only a pronounced suppression of growth processes in the first, but also certain alterations in the second plant growth generations (table 3).

TABLE 2. EFFECTS OF COMBINED RED (RL) AND FAR-RED LIGHT ON THE PD REACTION OF LETTUCE SEEDS IN INTERNAL AND EXTERNAL CONTAINERS ABOARD THE "KOSMOS-936" SATELLITE (NUMBER OF SEEDS GERMINATING IN 48 HOURS)

Version	Light action	Control	Flight	Flight % of control
Internal container	RL+FR	6.67±0.9	7.9±0.5	118.4
	FR+RL	11.0±1.3	10.7±1.4	97.3
External container	RL+FR	8.6±0.9	3.0±0.3	31.8
	FR+RL	9.2±1.1	5.4±0.7	58.7

Considering that the PD reaction is already reflected in initial growth processes, we took as a working assumption Mohr's hypothesis of gene activation by phytochrome Φ_{730}. According to Mohr's hypothesis/98 [6], Φ_{730} activates "potentially active genes" (transforming them into active ones), resulting in synthesis of the respective enzymes.

TABLE 3. EFFECTS OF PD REACTION ON GROWTH OF LETTUCE PRIMARY ROOT IN SECOND GENERATION AFTER SEED EXPOSURE ABOARD "SALYUT-6" ORBITAL SPACE STATION

Day of plant growth	Without red light			With red light		
	Control	Flight	% of control	Control	Flight	% of control
Day 6	7.10±0.4	7.91±0.3	106	6.17±0.4	6.19±0.2	105
Day 7	9.32±0.6	13.5±0.5	145	9.83±0.5	9.03±0.1	92
Day 8	13.50±0.6	16.8±0.7	124	15.10±0.5	12.50±1.0	83
Day 9	15.10±0.3	20.30±0.6	134	17.30±0.8	15.40±0.4	89

The effect of red light had been found in the very earliest plant growth and development periods, when the developing seed begins to show proliferation. During the growth process, the phytochrome system acts to trigger the proliferation of primary meristem tissue [15], bringing the cell out of a dormant state, which must be associated with derepression of a certain area of the cell genome. As Kuzin points out [16], gene activation affects enzyme synthesis, transcription, RNA synthesis, and all subsequent vital reactions in plant development.

Conclusions. It has been established that the phytochrome-dependent (PD) reaction can be used as a criterion for assessing the effects of space flight factors (SFF) on the lettuce plant.

It has been found that the PD reaction in air-dry lettuce seeds is suppressed after space flight. This appears most markedly when the seeds are exposed to open space (screen thickness 0.02 g/cm^2). SFF have been shown to affect both $\Phi_{660}D$ and $\Phi_{730}D$ reactions of lettuce seeds.

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